D3 concl'd

31. (twice amended) The method of claim 27, wherein the enhanced protein has a Km for $CO_2$ that is less than that of proteins encoded by the plurality of polynucleotide species, to an extent that is statistically significant.

32. (twice amended) The method of claim 27, wherein the enhanced protein has a Km for $O_2$ that is greater than that of proteins encoded by the plurality of polynucleotide species, to an extent that is statistically significant.

D4

37. (twice amended) The method of claim 36, wherein the sequence encoding the enhanced protein and the selectable marker gene are flanked by an upstream flanking recombinogenic sequence having sufficient sequence identity to a chloroplast genome sequence to mediate efficient recombination and a downstream flanking recombinogenic sequence having sufficient sequence identity to a chloroplast genome sequence to mediate efficient recombination.

These amendments are made without prejudice and are not to be construed as abandonment of the previously claimed subject matter or agreement with any objection or rejection of record. In accordance with the requirements of 37 C.F.R. § 1.121, a marked up version showing the changes to the claims, is attached herewith as Appendix A. For the Examiner's convenience, a complete claim set of the currently pending claims is also submitted herewith as Appendix B.

## REMARKS

### *1. Amendments to the specification*

The specification has been amended to change the term "velocity" to "maximal velocity" when used in reference to the maximal velocity (i.e., $V_{max}$) of a Rubisco catalyzed reaction. The amendment does not constitute new matter since it merely entails clarification of the language so as to render it more consistent with standard nomenclature. It is well known in the field of biochemistry that "maximal velocity", or $V_{max}$, is one of the two most fundamental kinetic constants used to quantify enzymatic catalytic activity (the other being the Michaelis constant, or $K_M$). One of skill in the art would undoubtedly recognize that the terms "velocity of carboxylation" and "velocity of oxygenation" as used in the specification to define the carboxylation specificity factor of an enzyme refer to "maximal velocity of carboxylation" and "maximal velocity of oxygenation." Indeed, carboxylation specificity factor is explicitly defined in the Spreitzer reference in terms of

"maximal velocity" – the definition is otherwise identical to that provided in the instant specification.

*2. Amendments to the claims*

Claims 27-32 and 37 have been amended to recite enhancement of activity "to an extent that is statistically significant." The term "statistically significant" is used throughout the specification, and is explicitly defined at page 29, lines 4-8.

Claims 29 and 30 were amended to recite "maximal velocity" rather than "velocity." The term "maximal velocity" is more standard in the field and does not constitute new matter, as discussed above in context of the corresponding amendment of the specification.

*3. 35 U.S.C. § 112, ¶ 1 Rejection*

Claims 27-37 were rejected under 35 U.S.C. § 112, ¶ 1 as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors were in possession of the claimed invention at the time the application was filed. Applicants respectfully traverse this rejection.

In particular, the Examiner found that claim 27 defines the ultimate product of the claimed method, i.e., a polynucleotide encoding a protein having "enhanced carboxylation activity," solely in terms of function and not in terms of its chemical and physical properties, and that this functional definition did not satisfy the written description requirement. The Examiner further found the same fault with the definition of the plurality polynucleotides species in terms of their ability to encode a protein having Rubisco activity. In support of this position, the Examiner cites *University of California v. Eli Lilly and Co.* 43 USPQ2d 1398 (1997) for the proposition that "[i]t is well settled law that to define a product, such as a nucleic acid, not by its chemical or physical properties, but in terms of its functional characteristics, does not constitute an adequate written description of the product."

Applicants respectfully submit that the rejection is based upon a misapplication of *Eli Lilly* to the facts of this case. In particular, the claims at issue in *Eli Lilly* claimed biological **compositions of matter**, i.e., nucleic acid vectors and microorganisms. 43 USPQ2d at 1405. The court in *Eli Lilly* held that "[i]n **claims to genetic material**, however, a generic statement such as "vertebrate insulin cDNA" or "mammalian insulin cDNA," without more, is not an adequate written description of the genus because it does not distinguish the claimed genus from others, except by

function." A full reading of *Eli Lilly* makes clear that this holding is limited by the facts of the case to the situation where biological material itself is being claims, i.e., composition of matter claims. There were no **method claims** at issue in *Eli Lilly*, method claims were not discussed or considered, and the case should not be read as establishing a heightened written description requirement for method claims.

It is important to bear in mind that in *Eli Lilly* statements regarding functional vs. strucural definition of biological material were made in the context of the validity of claims that claimed the biological materials per se. It would be a mistake to assume that those same statements would apply to the mere recitation of biological material in any claims, particularly in cases where it is a method that is being claimed rather than the biological materials themselves. As pointed out recently by the Federal Circuit in *EnzoBiochem v. Gen-Probe Inc.*, "[c]ompliance with the written description requirement is essentially a fact-based inquiry that will 'necessarily vary depending on the nature of the invention claimed.'" 296 F.3d 1316, 1324 (Fed. Cir. 2002) (citing In re DiLeone, 168 USPQ 592, 593 (CCPA 1971). The court went on to state that "[i]t is not correct, however, that all functional descriptions of genetic material fail to meet the written description requirement." Id.

In *Enzo*, the court was specifically asked to decide whether reference to a deposit of nucleotide sequence could adequately describe that sequence. Note that, as was the case in Eli Lilly, the claims at issue in Enzo claim the biological material *per se*, rather than simply referring to biological material in a method case as Applicant has done in the rejected claims. The court in *Enzo* found that in light of "***the history of biological deposits for patent purposes, the goals of the patent law, and the practical difficulties of describing unique biological materials in a written description***," reference in the specification to a deposit in a public depository constitutes an adequate written description. 296 F.3d at 1325.

Applying the three criteria articulated in *Enzo*, it is clear that with regard to the instant claimed invention, the description of a nucleotide sequence in terms of the function of a protein encoded by the sequence is sufficient to satisfy the written description requirement. The first articulated criterion looked to the ***historical treatment*** of the described subject matter for patent purposes. The US Patent Office, historically and up to the present day, has issued a large number of patents including method claims that recite polynucleotide sequences in terms of the function of a protein encoded by the sequence. For purposes of illustration Applicants will provide a few

examples, although the list could be extended *ad nauseam* should one be inclined to spend the time on the exercise.

Claim 1 of US Patent No. 6,406,855:

> 1. A method of identifying a cytokine variant with at least one desired property, the method comprising:
> (a) providing a mixture of nucleic acid subsequences of two or more parental polynucleotides, **wherein each parental polynucleotide** differs from at least one other parental polynucleotide in at least one nucleotide and **encodes at least one cytokine or fragment thereof**;
> (b) extending one or more of the nucleic acid subsequences with at least one polymerase to produce one or more **recombined polynucleotides that each encode one or more cytokine variants**;
> (c) expressing the one or more recombined polynucleotides to provide the one or more cytokine variants;
> (d) screening or selecting the one or more cytokine variants to identify at least one cytokine variant with the at least one desired property;
> (e) recovering at least one recombined polynucleotide encoding the at least one cytokine variant identified in step (d); and,
> (f) repeating (a)-(d) using the at least one recombined polynucleotide recovered in step (e) as at least one of the two or more parental polynucleotides of a repeated step (a).

Claim 1 of US Patent No. 6,406,910:

> 1. A method of making **a nucleic acid with a desired splicing phenotype**, the method comprising:
> providing a plurality of homologous nucleic acids, each comprising a plurality of insertion nucleic acid sequences, **wherein the plurality of insertion nucleic acid sequences comprise heterologous inteins**;
> recombining the plurality of homologous nucleic acids to produce a library of recombinant nucleic acids, and
> selecting the recombinant nucleic acids for a selected **recombinant nucleic acid which produces a desired mRNA or protein possessing the desired splicing phenotype** when the selected recombinant nucleic acid is expressed in a cell.

Claim 1 of US Patent No. 6,391,640:

> 1. A method of shuffling polynucleotides, the method comprising:
> (i) providing a plurality of polynucleotide variants to be shuffled;
> (ii) conducting a multi-cyclic polynucleotide extension process on at least partially annealed polynucleotide strands having sequences from the plurality of polynucleotide variants, the polynucleotide strands having

regions of similarity and regions of heterology with each other, and being at least partially annealed through the regions of similarity under conditions whereby one strand serves as a template for extension of another strand with which it is partially annealed, to generate a population of recombinant polynucleotides; and,
(iii) selecting or screening a recombinant polynucleotide from the population of recombinant polynucleotides for a desired property, **thereby producing an optimized recombinant polynucleotide having the desired property;**
**wherein the desired property comprises:**
**an elevated expression of the recombinant polynucleotide in a first cell relative to an expression level of one or more of the plurality of polynucleotide variants to be shuffled in the first cell**.

Claim 1 of US Patent No. 6,387,702:

1. A method of enhancing competence of a non-mammalian cell, comprising:
(1) **recombining at least first and second DNA segments from at least one gene conferring DNA competence**, the segments differing from each other in at least two nucleotides, to produce a library of recombinant genes;
(2) screening the library of recombinant genes for **at least one recombinant gene that confers enhanced competence in the non-mammalian cell relative to a wild-type form of the gene**;
(3) recombining at least a segment from the at least one recombinant gene with a further DNA segment from the at least one gene, the same or different from the first and second segments, to produce a further library of recombinant genes;
(4) screening the further library of recombinant genes for at least one further recombinant gene that confers enhanced competence in the non-mammalian cell relative to the at least one recombinant gene;
(5) repeating (3) and (4), as necessary, until the at least one further recombinant gene confers a desired level of enhanced competence in the non-mammalian cell; and
(6) introducing the at least one further recombinant gene to the non-mammalian cell, thereby conferring enhanced competence.

Claim 1 of US Patent No. 6,372,497:

1. A method of producing a recombined polynucleotide having a desired characteristic, comprising:
(a) providing a plurality of related-sequence double-stranded template polynucleotides, comprising polynucleotides with non-identical sequences;
(b) **providing a plurality of single-stranded nucleic acid fragments**

**capable of hybridizing to the template polynucleotides**;
(c) hybridizing single-stranded nucleic acid fragments to the template polynucleotides and extending the hybridized fragments on the template polynucleotides with a polymerase, thereby forming a plurality of sequence-recombined polynucleotides;
(d) subjecting the sequence recombined polynucleotides of step (c) to at least one additional cycle of recombination to produce further sequence-recombined poly-nucleotides; and,
(e) selecting or screening the further sequence-recombined polynucleotides for the desired characteristic.

Claim 1 of US Patent No. 6,440,668:

1. An in vitro method for producing **a polypeptide having an activity of interest,** comprising:
(a) blocking or interrupting a polynucleotide synthesis or amplification process with at least one member selected from the group consisting of UV light, one or more DNA adducts, DNA intercalating agents, and/or polymerase inhibitors or poisons, wherein the member blocks or interrupts polynucleotide synthesis or amplification, thereby providing a plurality of polynucleotides at various stages of synthesis;
(b) denaturing the plurality of polynucleotides obtained from step (b) to produce a mixture of single-stranded polynucleotides;
(c) incubating the mixture of single stranded polynucleotides with a polymerase under conditions which result in annealing of the single stranded polynucleotides at regions of identity between the single-stranded polynucleotides and which results in the synthesis of at least one mutagenized double stranded polynucleotide;
(d) repeating steps (c) and (d);
(e) generating at least one mutagenized double stranded polynucleotide that encodes a polypeptide; and
(f) screening the **at least one mutagenized polynucleotide that encodes a polypeptide to determine the polypeptide that possesses an activity of interest**.

Claim 1 of US Patent No. 6,335,179:

1. A process for providing a thermostable enzyme having improved enzyme activities as compared to a corresponding wild-type enzyme at lower temperatures comprising:
(a) subjecting to random mutagenesis at least one polynucleotide encoding an enzyme which is stable at a temperature of at least 60°C.; and
(b) screening mutants produced in (a) for a mutated enzyme or for a polynucleotide encoding a mutated enzyme, wherein the mutated enzyme is stable at a temperature of at least 60.degree. C. and has increased

enzyme activity at a lower temperature than that of the corresponding wild-type enzyme at its optimal temperature.

Note further that there is nothing in the MPEP or case law (particularly *Eli Lilly* and *Enzo*) that would require a structural definition of a polynucleotide sequence in a method claim. The US Patent Offices Written Description Guidelines, a response to Eli Lilly, can be found in section 2163 of the MPEP. A careful reading of these guidelines reveals that they do not impose any requirement of structural definition of a polynucleotide sequence in method claims. In fact, at several points the guidelines specifically point out that the requirement of structure does not apply to method claims. See, for example, the first sentence of MPEP 2163(II)(A)(3)(a)(i)(c)(1), which requires the Examiner to [d]etermine whether the application as filed described the complete structure ***(or acts of a process)*** of the claimed invention as a whole. (emphasis added)" Thus, the guidelines do not require a description of structure where the claimed invention is a process, but simply that the application as filed describes the acts of the process, i.e., the steps of the method. In the instant case all steps of the claimed method are described in the specification. In summary, the MPEP and case law do not require a recitation of structure for the instant claims, and it has been and continues to be the policy of the U.S. Patent Office to issue claims of this type that define biological sequences solely in terms of function.

The second criterion articulated by the court in *Enzo* is "the goals of the patent law." It is the goal of the patent law "to promote the Progress of Science and useful Arts, by securing for limited Times to Authors and Inventors the exclusive Right to their respective Writings and Discoveries." Section 8 of the US Constitution. 35 USC § 101 provides that "whoever invents or discovers and new or useful process . . . may obtain a patent therefor, subject to the conditions and requirements of this title." Thus, it is clearly the intent of the patent law to allow an inventor of a new and useful method to obtain patent claims commensurate in scope with the disclosed invention. The instant claimed invention provides a method of starting with one or more sequences encoding proteins with Rubsico carboxylation activity, shuffling the sequence, and screening to obtain a protein with enhanced carboxylation activity relative to the starting material. For any given parental sequences there will typically be many possible recombinant sequences having enhanced activity, and hence a large number of potential polynucleotide sequences that might result from practice of the invention.

The fact that the invention is applicable to a class of polynculeotides of diverse sequence should not prevent the inventor from receiving claims of a scope commensurate with the disclosure. To do so would rob the inventor of meaningful patent coverage, contrary to the goals of the patent law.

The third criterion articulated in *Enzo* is the practical difficulties of describing the relevant subject matter in a written description. This is an important factor with regard to the claimed invention. In light of the large number of Rubisco variants, and the even larger number of sequence shuffled variants with enhanced carboxylation activity that can be produced using the method of the claimed invention, it is impossible from a practical standpoint to define the sequences in terms of structure. In short, analysis of all three of the *Enzo* criteria weigh in favor of finding that recited polynucleotides are adequately defined to comply with the written description requirement.

Claims 36 and 37 were further rejected for failure to define the "marker gene" in terms of its chemical or physical properties. Thus, once again a method claim is being rejected for not providing structural definition of biological material used in the method. The arguments set forth above applies equally to the use of this term. That is, the claimed method can be practiced using any of a number of marker genes, as described in the specification. It is not any particular sequence that is important to the claimed invention, but rather the ability to function as a marker. In terms of historical treatment, it is and has been the accepted practice of the U.S. Patent Office is to issue method claims using the term "marker gene" without further structural definition. In fact, a search of the USPTO patent database on Oct 17, 2002 for the term "marker gene" in the claims turned up 435 hits. Many if not most of these do not include structural definition of the "marker gene." Some examples that have issued just this month include US patents 6,459,017; 6,458,594 6,458,592; 6,458,529 and 6,448,006.

For the reasons stated above, it is respectfully requested that all rejections under 35 U.S.C. § 112, ¶ 1 be withdrawn.

***4. 35 U.S.C. § 112, ¶ 2 Rejection***

Claims 27-27 were rejected under 35 U.S.C. § 112, ¶ 2 as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

In particular, the examiner finds the use of the terms "significantly enhanced" in claim 27, "higher" in claim 28, "greater than" in claims 29 and 32, and "less than" in claims 30 and 31 fail to

adequately set forth "metes and bounds" of the claim. Claims 27-32 have been amended so as to recite enhancments that are of a degree that is statistically significant. Withdrawal of this rejection is respectfully requested.

The non-standard usage of the term "velocity" in claim 30 was presumably the basis for the Examiner's rejection of this claim under 35 U.S. C. § 112, ¶ 2. As amended, the claim (and also claim 29) recites "maximal velocity," standard terminology in the field of biochemistry, as discussed above in connection with the amendments. Withdrawal of this rejection is respectfully requested.

*5. 35 U.S.C. § 103 Rejection*

Claims 27 was rejected under 35 U.S.C. § 103 as allegedly unpatentable over the combination of Minshull et al., Spreitzer, and Wolter et al. The Examiner asserts that it would have been obvious to have adapted the method of Minshull et al. so as to allow for the shuffling and screening of polynucleotide sequences that encode rubisco with desired properties as the gene for rubisco had not only been isolated and characterized and known to undergo shuffling naturally, but the gene product was of long interest in the art and the isolation of desirable mutant clones continued to be of much interest. Applicants respectfully traverse this rejection.

To establish a *prima facie* case of obviousness, there must be some suggestion or motivation to modify or combine the reference teachings. Furthermore, there must be a reasonable expectation of success, viewed in light of the prior art (MPEP 2142 and references cited therein).

As noted by the Examiner, none of the cited references explicitly teach or suggest applying the method of Minshull to the improvement of Rubisco. In the absence of an explicit suggestion or motivation to combine the reference, the Examiner asserts that the suggestion to combine the references is implicit in the references themselves and/or in the prior art. However, this assertion is effectively rebutted by the references themselves and the state of the art as generally accepted by the skilled artisan at the time the application was filed.

*A.* **The cited references teach away from the combination proposed by the Examiner**

It is improper to combine references where the references teach away from their combination. *In re Grasselli*, 218 USPQ 769, 779 (Fed. Cir. 1983). The Examiner asserts that Spreitzer suggests finding "desirable clones." However, a prior art reference must be considered in

its entirety, i.e., as a whole, including portions that would lead away from the claimed invention. See MPEP 2141.03 and references cited therein.

The teaching Spreitzer is discussed in the declaration of Dr. Genhai Zhu ('the Declaration"), an expert in the area of Rubisco. As pointed out by Dr. Zhu in paragraph 8 of the Declaration, the section of Spreitzer cited by the Examiner (*Screening for Mutations*, page 416-18) is discussing screening for ***lethal*** Rubisco mutations, not Rubisco mutants having ***enhanced*** carboxylation activity as recited in the claims. The opening sentence of the *Screening for Mutations* section is "[e]ven though ***favorable Rubisco mutations cannot be selected*** directly, lethal Rubisco mutations would also be of value." The basis for Spreitzer's statement that mutants having enhanced carboxylation activity cannot be selected for directly can be found in the preceding section of the paper entitled *Selection for a Better Enzyme*, pages 415-16. In that section, Spreitzer reviews a number of unsuccessful attempts that have been made to screen for improved Rubisco mutants. In an attempt by Somerville and Ogren, $5 \times 10^6$ plants were screened without finding a single one expressing an improved Rubisco. In a similar experiment, Spreitzer et al. screened greater than $1 \times 10^9$ mutagenized *C. reinhardtii* cells without finding a single Rubisco with improved catalytic constants. Pierce et al. are described as taking a more molecular approach, wherein their would be strong selection for a better enzyme under normal atmosphere – once again, no mutation was identified that improves Rubisco. Spreitzer concludes by stating that it will take more than a single amino acid substitution to make a better Rubisco, but if two specific amino acid substitutions are required simultaneously to make a better Rubisco, more than $1 \times 10^{16}$ cells would need to be subjected to selection. He notes ruefully that there are fewer cells than this living in all the *Chlamydomonas* laboratories on the planet. Clearly, when read in its entirety Spreitzer teaches away from any attempt to screen a population of Rubisco variants for one having enhanced activity.

***B. There was no reasonable expectation of successfully combining the cited references***

The teaching of Spreitzer is entirely consistent with what was generally understood by those of skill in the art prior to the present invention, *i.e.*, that is spite of the long-felt need for a Rubisco with enhanced carboxylation specificity, there was no reasonable expectation that screening for an enhanced Rubisco mutant would be successful. Without this reasonable expectation of success, there can be no *prima facie* showing of obviousness.

As evidence of the general lack of reasonable expectation of success, the Examiner's attention is directed to the Declaration and the references cited therein. As stated by Dr. Zhu in

paragraph 7 of the Declaration, one of ordinary skill in the art at the time of invention would not have been motivated to apply the method of Minshull to obtain a polynucleotide encoding a Rubisco variant having enhanced Rubisco carboxylation activity. In November of 1998 (the priority date for this application), the general consensus in the field was that it would not be reasonable to expect success in screening a library of Rubisco variants for one with enhanced carboxylation activity. This view was based on the experience of those working in this area, who for many years had attempted to engineer and improve Rubisco. Their lack of success, and the very nature of the enzyme itself and the catalyzed reaction, strongly suggested that this approach would probably not be successful using the genetic engineering and screening technologies available at that time.

In support of Dr. Zhu's opinion, he points to two recent review articles, copies of which are enclosed herewith. One of these references, discussed in paragraphs 9 and 10 of the Declaration, is a review article entitled "Genetic Engineers Aim to Soup Up Crop Photosynthesis" that appeared in an edition of *Science* that published in January 1999. (Mann, C.C., (1999) *Science* 283:314-16). Near the end of page 314 the author states that the quest for a better Rubisco is a Holy Grail in plant biology, but "[d]espite more than 20 years of effort, the hopes have not yet paid off."

The author of this review article does find some basis for hope in recent advances in molecular biology and the unexpected discovery of more efficient Rubisco in red algae. "In what may be the ***most ambitious genetic engineering project ever tried***, laboratories across the world are trying to improve the Rubisco in food crops by either replacing the existing enzyme with the red algae form or bolting on what could be thought of as molecular superchargers." (first partial paragraph on page 315, emphasis added). With regard to the strategy of replacing the existing enzyme with the red algae form, an expert in the field is quoted as stating that "[i]f it can be done, it would really be amazing." (first full paragraph on page 316). The leader of a team attempting that approach admits that "[i]t may not work" and that he hopes to see results in "about 10 years." (second full paragraph on page 316). The other approach cited (" bolting on molecular superchargers") would involve introducing the $C_4$ cycle into $C_3$ plants such as rice. The author states that "the project may well be the most fundamental genetic alteration that humankind has ever tried in any organism. 'Don't hold your breath'." (top of the middle column of page 316). The author goes on to detail the many potential pitfalls to this approach that suggest a low probability of success.

The review article also discusses rational, structure-based approaches to the problem of improving Rubsico that have been employed in recent years. (See the first paragraph of the section entitled "A better Rubisco," right column of page 315) Researchers spent years determining molecular structures of Rubsicos (the spinach and cyanobacterial forms of the enzyme are specifically discussed) and comparing the structures in an attempt to rationally engineer an improved Rubisco. These attempts were unsuccessful, dismaying many researchers and leading at least one group to disband (first sentence of paragraph bridging pages 315-316).

Dr. Zhu points out that, significantly, the article does not suggest or even mention using an approach that would involve screening for an improved Rubisco variant. This implies that the author of the review viewed the likelihood of this approach succeeding to be even less probable than the approaches he does discuss, all of which he concedes are long-shots in terms of likelihood of success. This view is entirely consistent with the consensus opinion of those working in the field at the time, i.e., that an attempt to obtain an enhanced Rubisco by the screening of a library of variants would not be likely to succeed, and thus it would be worth trying alternate approaches that are technically challenging and not likely to succeed.

In the second review article cited by Dr. Zhu in paragraph 11 of the Declaration (Gewolb, J. (2002) *Science* 295:258-59, attached as Exhibit C), the author states that "[d]ozens of research groups have [tried to alter Rubisco] to improve the efficiency of photosynthesis, but none so far have succeeded." Dr. Zhu notes that in spite of there being a long-felt need for an improved Rubisco, prior to the instant claimed invention the attempts have met with failure.

In a previous Office Action, the Examiner himself apparently made reference to the low expectation of success for improving Rubisco. In the Office Action dated April 10, 2001, the Examiner states that "much interest exists in the identification of mutant genes that encode enzymes with enhanced properties. In so far as the prior art teaching the identification of such mutated Rubisco enzymes, there is very limited development in the relevant art." (page 5, section entitled The State of the Prior Art). This statement is consistent with the opinion of Dr. Zhu and the above-cited review articles, and suggest that the Examiner is aware of the fact that many have attempted to improve Rubisco using a variety of approaches, but prior to this invention there has been little or no success.

In summary, the record clearly supports Applicants' position that in this case there was no reasonable expectation that the cited references could be combined successfully. Spreitzer teaches

that previous attempts at screening for improved Rubisco variants have been unsuccessful and postulates that further screening-based approaches would likely also fail. Recent review articles, published well after the references cited by the Examiner, do not even mention a screening approach to identifying an improved Rubsico, reflecting the view of those in the field that such attempts would likely be futile. Instead, the 1999 *Science* article discusses rational design approaches, the introduction of cyanobacterial enzyme into plants, and the introduction of a $C_4$ pathway into a $C_3$ plant. The rational design approach has thus far proven futile, while the success of the other two proposed approaches is acknowledged to be a long shot. Surely, with the high interest in generating a better Rubsico and the lack of any truly promising strategy to achieve this result, someone of skill in the art would have at least suggested combining the cited references if they had viewed the combination as being likely to succeed.

***3. The test for obviousness is not "obvious to try"***

It is clearly the case that "obvious to try" is not the standard under Section 103 (MPEP 2145(X)(B) and references cited therein). Regardless of whether one of skill in the art might have found it obvious to try to apply the general teachings of Minshull to the specific problem of improving Rubisco, absent a reasonable expectation of success there would have been insufficient motivation to combine the references.

***4. The Wolter reference***

The Examiner states that Wolter et al. "disclose the shuffling of rubisco gene [sic] during evolution." Dr. Zhu discusses this assertion in paragraph 12 of the Declaration. Dr. Zhu states that Wolter et al. analyzed the sequences of known Rubisco genes and postulate that the structure of small subunit genes of $RbuP_2$ is the result of two counteracting processes working sequentially during evolution. First, introns were introduced before or during exon shuffling, adding new domains for new functions. Later, these introns were lost stepwise, leading to a more streamlined gene structure." (quoting the final paragraph). It is not the shuffling of exons that is noteworthy, but rather the introduction and stepwise loss of introns during the process. It is known in the field that "exon shuffling" is a general mechanism thought to occur during evolution, *i.e.,* it is not something specific or noteworthy with regard to Rubsico. Dr. Zhu concludes that those working in the area of Rubisco enzymology would regard the teaching of Wolter as essentially irrelevant with respect to the question of whether it would be obvious to apply the teaching of Minshull to the problem of generating an improved Rubisco.

## CONCLUSION

In view of the foregoing, Applicants believes all claims now pending in this application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If a telephone conference would expedite prosecution of this application, the Examiner is invited to telephone the undersigned at (650) 298-5884.

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Respectfully submitted,

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## APPENDIX A

## "MARKED UP" CLAIMS ILLUSTRATING THE AMENDMENTS MADE TO THE CLAIMS OF 09/437,726 WITH ENTRY OF THIS AMENDMENT

27. (amended) A method for obtaining an isolated polynucleotide comprising a sequence encoding a protein having Rubisco carboxylation activity, the method comprising:

recombining a plurality of parental polynucleotide species encoding at least one protein having Rubisco carboxylation activity under conditions suitable for sequence shuffling to form a resultant library of sequence-shuffled polynucleotides;

transferring said library into a plurality of host cells, thereby forming a library of transformants wherein sequence-shuffled Rubisco polynucleotides are expressed;

identifying at least one transformant from said library that expresses an enhanced [a] protein having a Rubisco carboxylation activity that is [significantly] enhanced to an extent that is statistically significant relative to the Rubisco carboxylation activity of proteins encoded by the plurality of parental polynucleotide species, wherein the identified transformant contains a polynucleotide comprising a sequence encoding the enhanced protein [having an enhanced Rubisco carboxylation activity]; thereby obtaining a polynucleotide comprising a sequence encoding the enhanced protein [having an enhanced Rubisco carboxylation activity].

28. (twice amended) The method of claim 27, wherein the enhanced protein [encoded protein having an enhanced Rubisco carboxylation activity] has a higher carboxylation specificity factor, to an extent that is statistically significant, than proteins encoded by the plurality of polynucleotide species.

29. (twice amended) The method of claim 27, wherein the enhanced protein [encoded protein having an enhanced Rubisco carboxylation activity] has a maximal velocity of carboxylation that is greater than that of proteins encoded by the plurality of polynucleotide species, to an extent that is statistically significant.

30. (twice amended) The method of claim 27, wherein the enhanced protein [encoded protein having an enhanced Rubisco carboxylation activity] has a maximal velocity of oxygenation that is less than that of proteins encoded by the plurality of polynucleotide species, to an extent that is statistically significant.

31. (twice amended) The method of claim 27, wherein the enhanced protein [encoded protein having an enhanced Rubisco carboxylation activity] has a Km for $CO_2$ that is less than that of proteins encoded by the plurality of polynucleotide species, to an extent that is statistically significant.

32. (twice amended) The method of claim 27, wherein the enhanced protein [encoded protein having an enhanced Rubisco carboxylation activity] has a Km for $O_2$ that is greater than that of proteins encoded by the plurality of polynucleotide species, to an extent that is statistically significant.

37. (twice amended) The method of claim 36, wherein the sequence encoding the enhanced protein [a protein having Rubisco carboxylation activity] and the selectable marker gene are flanked by an upstream flanking recombinogenic sequence having sufficient sequence identity to a chloroplast genome sequence to mediate efficient recombination and a downstream flanking recombinogenic sequence having sufficient sequence identity to a chloroplast genome sequence to mediate efficient recombination.

## APPENDIX B

## CLAIMS PENDING IN USSN 09/437,726 WITH ENTRY OF THIS AMENDMENT

27. (amended) A method for obtaining an isolated polynucleotide comprising a sequence encoding a protein having Rubisco carboxylation activity, the method comprising:

recombining a plurality of parental polynucleotide species encoding at least one protein having Rubisco carboxylation activity under conditions suitable for sequence shuffling to form a resultant library of sequence-shuffled polynucleotides;

transferring said library into a plurality of host cells, thereby forming a library of transformants wherein sequence-shuffled Rubisco polynucleotides are expressed;

identifying at least one transformant from said library that expresses an enhanced protein having a Rubisco carboxylation activity that is enhanced to an extent that is statistically significant relative to the Rubisco carboxylation activity of proteins encoded by the plurality of parental polynucleotide species, wherein the identified transformant contains a polynucleotide comprising a sequence encoding the enhanced protein; thereby obtaining a polynucleotide comprising a sequence encoding the enhanced protein.

28. (twice amended) The method of claim 27, wherein the enhanced protein has a higher carboxylation specificity factor, to an extent that is statistically significant, than proteins encoded by the plurality of polynucleotide species.

29. (twice amended) The method of claim 27, wherein the enhanced protein has a maximal velocity of carboxylation that is greater than that of proteins encoded by the plurality of polynucleotide species, to an extent that is statistically significant.

30. (twice amended) The method of claim 27, wherein the enhanced protein has a maximal velocity of oxygenation that is less than that of proteins encoded by the plurality of polynucleotide species, to an extent that is statistically significant.

31. (twice amended) The method of claim 27, wherein the enhanced protein has a Km for $CO_2$ that is less than that of proteins encoded by the plurality of polynucleotide species, to an extent that is statistically significant.

32. (twice amended) The method of claim 27, wherein the enhanced protein has a Km for $O_2$ that is greater than that of proteins encoded by the plurality of polynucleotide species, to an extent that is statistically significant.

33. (amended) The method of claim 27, wherein the plurality of parental polynucleotide species encodes at least one Rubisco Form I L subunit.

34. (amended) The method of claim 27, wherein the plurality of parental polynucleotide species encodes at least one Rubisco Form I S subunit.

35. (amended) The method of claim 27, wherein the plurality of parental polynucleotide species encodes at least one Rubisco Form II subunit.

36. (amended) The method of claim 27 further comprising a selectable marker gene which affords a means of selection when expressed in chloroplasts.

37. (twice amended) The method of claim 36, wherein the sequence encoding the enhanced protein and the selectable marker gene are flanked by an upstream flanking recombinogenic sequence having sufficient sequence identity to a chloroplast genome sequence to mediate efficient recombination and a downstream flanking recombinogenic sequence having sufficient sequence identity to a chloroplast genome sequence to mediate efficient recombination.